The cross-resistance patterns and biochemical characteristics of an imidacloprid-resistant strain of the cotton aphid

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(Received March 31, 2014; Accepted January 8, 2015)

The imidacloprid-resistant strain of Aphis gossypii (RF60) was further selected with imidacloprid to establish the more resistant strain (RF75). After the selection for 15 generations, the resistance ratio relative to the susceptible strain increased from 66.60 to 72.60. The RF75 strain showed different levels of cross-resistance to all the insecticides tested. Synergistic and metabolic enzyme assays suggested that carboxylesterase and cytochrome P450 may play important roles in the imidacloprid resistance. © Pesticide Science Society of Japan

Keywords: Aphis gossypii, cross-resistance, cytochrome P450, carboxylesterase, imidacloprid, synergism.

Introduction

The cotton aphid, Aphis gossypii (Glover) (Homoptera: Aphididae), is widely considered to be one of the most cosmopolitan and significant crop pests. It can cause both direct and indirect damage by feeding on plants, by honeydew contamination or by transmitting a large number of plant viruses.1,2) Due to the extensive and frequent use of pesticides, this pest has developed a high level of resistance to some classes of insecticides and has become an unmanageable pest. Resistance patterns have been well studied for several types of insecticides such as organophosphates, carbamates, and pyrethroids.3–5)

Since the introduction of imidacloprid in the early 1990s, neonicotinoids have demonstrated outstanding potency for the control of several important pests, including aphids.6) Due to the long-lasting preventative effects, relatively low toxicity to non-target organisms and diverse application methods, neonicotinoids have become one of the most widely used insecticides. At the same time, due to its unique mechanism of action, acting as an agonist targeting the nicotinic acetylcholine receptor (nAChR) in the insect central nervous system, neonicotinoids have played important roles in overcoming the challenges of insect resistance to earlier classes of insecticides.7,8)

Nevertheless, resistance to neonicotinoids has been reported in some pests.9–11) In addition, cross-resistance between neonicotinoids and other groups of insecticides has been investigated in several pests.12,13) Neonicotinoid insecticides, including imidacloprid, have replaced pyrethroids, chlorinated hydrocarbons, organophosphates, carbamates, and several other classes of insecticides and have become the primary insecticide for A. gossypii control in China with high effectiveness.14) However, increasingly, problems of A. gossypii resistance to imidacloprid have been reported.15,16) Additionally, previous reports revealed that metabolic enzymes, including esterases, glutathione S-transferase, and P450-monoxygenases, play important roles in resistance to insecticides and cross-resistance development in some insects.17–19)

Though reports of cross-resistance between imidacloprid and other classes of insecticides are not uncommon, systematic research regarding cross-resistance to conventional insecticides in A. gossypii with high levels of resistance to imidacloprid is rare, especially in China. In this study, the strain of Aphis gossypii with a high resistance to imidacloprid was further selected with the same insecticide to generate a more resistant strain. Using the more resistant strain, the levels of cross-resistance to different groups of insecticides, including neonicotinoids, as well as the biochemical characteristics, were studied.

Materials and Methods

1. Insects

The susceptible strain (SS) of A. gossypii was collected from hibiscus plants (Hibiscus rosa sinensis L.) in Taian, Shandong, China, in 2011 and was reared on cotton (Gossypium hirsutum L.) seedlings in a greenhouse without any insecticide exposure. The cotton seedlings were grown in a nutrient solution consisting of FeSO4, KH2PO4, MgSO4, KNO3 and Ca(NO3)2.21) The resistant strain selected with imidacloprid for 60 generations (RF60 strain)20) was further selected with the same insecticide for 15 generations in the laboratory to generate the more imidacloprid-resistant strain (RF75 strain). After the selection for 15 generations, the resistance ratio relative to the SS strain increased from 66.60 to 72.60. All test insects were reared in the laboratory at 25±1°C, 60–70% relative humidity and a 16:8 hr (light:dark) photoperiod.

Note

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Published online April 22, 2015

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2. Chemicals
All insecticides used were commercial formulations. Imidacloprid (purity 95.3%) and endosulfan (purity 94.0%) were obtained from the Jiangsu Changzhou Chemical Co., Ltd. (Jiangsu, China). Acetamiprid (purity 95.8%), thiamethoxam (purity 96.0%), dinotefuran (purity 95.8%), omethoate (purity 76.0%) and lambda-cyhalothrin (purity 97.1%) were obtained from the Shandong Liaherd Chemical Industry Co., Ltd. (Shandong, China). Clothianidin (purity 95.0%), nitepyratm (purity 95.7%) and pymetrozine (purity 98.8%) were obtained from the Shandong Jingpeng Bio-Pesticide Co., Ltd. (Shandong, China). Carbosulfan (purity 85.0%) was obtained from the Shandong Huayang Technology Co., Ltd. (Shandong, China).

Three synergists, triphenyl phosphate (TPP; analytical grade), diethyl maleate (DEM; analytical grade), and piperonyl butoxide (PBO; 98.0%) were obtained from the Shanghai Chemical Reagent Co., Ltd. (Shanghai, China). Bovine serum albumin (BSA), glutathione (GSH), 1-chloro-2,4-dinitrobenzene (CDNB), p-nitroanisole (p-NA), nicotinamide adenine dinucleotide phosphate (NADPH), analytical grade 1-naphthyl acetate (α-NA), Coomassie Brilliant Blue G-250, and p-nitrophenol (PNP) were obtained from the Shanghai Chemical Reagent Co., Ltd. (Shanghai, China). The other chemicals used in the experiments were of analytical grade.

3. Bioassays
The toxicity of the insecticides for the different strains of A. gossypii was determined using a dipping method. A stock of insecticide was dissolved in aceton containing 0.5% Tween-80 as an emulsifier. Then each stock was diluted to at least five concentrations with distilled water prior to the bioassay. Cotton leaves with at least 50 apterous adults A. gossypii were dipped into the freshly prepared solutions for 3–5 sec; each concentration had six replications. Controls used water containing Tween-80 and acetone alone. The total number of cotton aphids was recorded when the leaves were completely dry. Following drying, the cotton leaves were placed in an incubator at 25°C and the supernatant was collected as the enzyme source. The reaction mixture consisted of GSH (50 mM), CDNB (30 mM), and an aliquot of the enzyme solution (0.2 mL). The activity of GST was determined using the extinction coefficient of 9.6 mM⁻¹ cm⁻² for CDNB. Absorbance changes were recorded at 340 nm after 5 min in a water bath at 25°C.

Cytotoxic P450-mediated O-demethylation activity toward p-nitroanisole (PNOD) was tested according to the method reported by Yu and Nguyen with minor modifications. A total of 200 apterous adults were homogenized in phosphate buffer (66 mM, pH 7.0) at 25°C. A total of 100 apterous adults were homogenized in phosphate buffer (5 mL, 40 mM, pH 7.0) on ice. The homogenate was centrifuged at 10,000 rpm for 15 min at 4°C and the supernatant was collected as the enzyme source. The reaction mixture consisted of GSH (50 mM), CDNB (30 mM), and an aliquot of the enzyme solution (0.2 mL). The activity of GST was determined using the extinction coefficient of 9.6 mM⁻¹ cm⁻² for CDNB. Absorbance changes were recorded at 340 nm after 5 min in a water bath at 25°C.

4. Toxicity tests with synergists
According to the methods of Mu, three synergists were dissolved to 10,000 mg/L with acetone and then diluted to 50 mg/L with distilled water. Each insecticide stock solution was diluted to five concentrations using the 50 mg/L synergist solutions. The toxicity of the insecticide with the synergists PBO, TPP, and DEM, inhibitors of cytochrome P450, carboxylesterase (CarE), and glutathione S-transferase (GST), respectively, was evaluated using the bioassay method described previously. Controls used water containing acetone and the synergists.

5. Metabolic enzyme assays
The total protein content was measured according to the Bradford method using Coomassie Brilliant Blue G-250 with bovine serum albumin as the standard.

CarE activity toward α-NA was determined by measuring the optical density at 600 nm (OD600) according to the method of van Asperen with minor modifications. A total of 100 apterous adults were homogenized in phosphate buffer (5 mL, 40 mM, pH 7.0) on ice. The homogenate was centrifuged at 10,000 rpm for 15 min at 4°C and the supernatant was collected as the enzyme source. A standard curve was prepared with α-naphthol. The reaction mixture included: α-NA (0.3 mM), eserine (0.1 mM), phosphate buffer (40 mM, pH 7.0), a chromogenic agent and an enzyme solution (0.1–0.5 mL).

For the determination of GST activity, the procedure of Habig et al. was adopted with CDNB as the substrate in phosphate buffer (66 mM, pH 7.0) at 25°C. A total of 100 apterous adults were homogenized in phosphate buffer (5 mL, 40 mM, pH 7.0) on ice. The homogenate was centrifuged at 10,000 rpm for 15 min at 4°C and the supernatant was collected as the enzyme source. The reaction mixture consisted of GSH (50 mM), CDNB (30 mM), and an aliquot of the enzyme solution (0.2 mL). The activity of GST was determined using the extinction coefficient of 9.6 mM⁻¹ cm⁻² for CDNB. Absorbance changes were recorded at 340 nm after 5 min in a water bath at 25°C.

6. Data analysis
The experimental data were statistically analyzed by probit analysis using the SPSS data processing system. Abbott’s formula was used to calculate the correction mortality for each probit analysis. The resistance ratios were calculated as the LC50 value of the resistant strain/LC50 value of the susceptible strain. Synergistic ratios were calculated as the LC50 value without the synergist/LC50 value with the synergist. All data for enzyme activities were expressed as the mean ± standard error (SE). Further statistical analysis was performed using a one-way ANOVA, and comparisons of the means were made using Fisher’s LSD test and a t-test (p<0.05).
Results

1. Cross-resistance to other insecticides

The cross-resistance pattern for different groups of insecticides was determined in the RF75 strain with the SS strain as the control. The results indicated that the RF75 strain had developed varying degrees of resistance to all the insecticides tested (Table 1). Compared with the SS strain, the RF75 strain showed cross-resistance to pymetrozine (5.28-fold), omethoate (5.90-fold), lambda-cyhalothrin (7.77-fold), carbosulfan (8.40-fold), endosulfan (9.18-fold), dinotefuran (10.00-fold), clothianidin (11.92-fold), thiamethoxam (14.47-fold), nitenpyram (14.85-fold), and acetamiprid (15.30-fold).

2. Toxicity tests with the synergists

The synergistic effects of PBO, TPP, and DEM with imidacloprid against the SS and RF75 strains are shown in Table 2. Synergism for PBO and TPP was observed in the RF75 strain (3.49-fold and 1.85-fold, respectively), but there were no obvious effects on the SS strain (1.21-fold and 1.21-fold, respectively). No synergistic effects were observed for DEM in either strain.

3. Metabolic enzyme activities

The SS and RF75 strains were analyzed for the activities of PNOD, CarE, and GST; the results are shown in Table 3. These results indicated that the activity of PNOD was significantly higher in the RF75 strain than in the SS strain (Ratio = 4.59).

Table 1. Toxicities of different insecticides to RF75 and SS strains of A. gossypii

| Insecticides  | Strains | Slope±SE | LC50 (µg/mL) | 95% F.L | Resistance ratio 
<table>
<thead>
<tr>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Imidacloprid</td>
<td>SS</td>
<td>3.62±0.84</td>
<td>0.35 (0.31–0.39)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>RF75</td>
<td>2.10±0.13</td>
<td>25.41 (22.80–28.80)</td>
<td>72.60</td>
<td></td>
</tr>
<tr>
<td>Acetamiprid</td>
<td>SS</td>
<td>1.48±0.21</td>
<td>1.68 (0.15–4.01)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>RF75</td>
<td>4.28±0.38</td>
<td>25.70 (22.80–28.30)</td>
<td>15.30</td>
<td></td>
</tr>
<tr>
<td>Nitenpyram</td>
<td>SS</td>
<td>2.67±0.67</td>
<td>3.36 (3.30–3.80)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>RF75</td>
<td>2.77±0.36</td>
<td>49.90 (42.70–56.80)</td>
<td>14.85</td>
<td></td>
</tr>
<tr>
<td>Thiamethoxam</td>
<td>SS</td>
<td>3.30±0.35</td>
<td>1.88 (1.52–2.20)</td>
<td>1.00</td>
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<tr>
<td></td>
<td>RF75</td>
<td>1.41±0.10</td>
<td>27.20 (20.60–40.10)</td>
<td>14.47</td>
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<tr>
<td>Clothianidin</td>
<td>SS</td>
<td>3.32±0.39</td>
<td>2.14 (1.68–2.53)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>RF75</td>
<td>1.92±0.30</td>
<td>25.50 (20.30–30.70)</td>
<td>11.92</td>
<td></td>
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<tr>
<td>Dinotefuran</td>
<td>SS</td>
<td>2.39±0.24</td>
<td>1.48 (1.17–1.74)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>RF75</td>
<td>2.99±0.29</td>
<td>14.80 (12.50–16.80)</td>
<td>10.00</td>
<td></td>
</tr>
<tr>
<td>Omethoate</td>
<td>SS</td>
<td>1.85±0.12</td>
<td>11.20 (10.00–12.90)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>RF75</td>
<td>1.79±0.12</td>
<td>66.10 (53.50–86.70)</td>
<td>5.90</td>
<td></td>
</tr>
<tr>
<td>Lambda-cyhalothrin</td>
<td>SS</td>
<td>1.64±0.12</td>
<td>14.80 (12.80–17.70)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>RF75</td>
<td>2.60±0.15</td>
<td>115.00 (96.70–141.00)</td>
<td>7.77</td>
<td></td>
</tr>
<tr>
<td>Carbosulfan</td>
<td>SS</td>
<td>3.01±0.28</td>
<td>7.42 (6.37–8.39)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>RF75</td>
<td>2.63±0.34</td>
<td>62.30 (50.70–72.80)</td>
<td>8.40</td>
<td></td>
</tr>
<tr>
<td>Pymetrozine</td>
<td>SS</td>
<td>3.40±0.40</td>
<td>2.18 (1.72–2.57)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>RF75</td>
<td>3.49±0.34</td>
<td>11.50 (8.87–13.80)</td>
<td>5.28</td>
<td></td>
</tr>
<tr>
<td>Endosulfan</td>
<td>SS</td>
<td>3.66±0.36</td>
<td>6.71 (4.78–8.23)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>RF75</td>
<td>3.53±0.23</td>
<td>61.60 (46.00–76.40)</td>
<td>9.18</td>
<td></td>
</tr>
</tbody>
</table>

a) Fiducial limits (probit analysis using SPSS data processing system). b) Resistance ratio = LC50 value of the RF75 strain / LC50 value of the SS strain.

Table 2. Synergistic effect of TPP, PBO and DEM on the toxicity of imidacloprid to SS and RF75 strains of A. gossypii

<table>
<thead>
<tr>
<th>Strain</th>
<th>Insecticide/synergist</th>
<th>Slope±SE</th>
<th>LC50 (µg/mL)</th>
<th>95% F.L</th>
<th>SR</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS</td>
<td>Imidacloprid</td>
<td>3.62±0.84</td>
<td>0.35 (0.31–0.39)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Imidacloprid+PBO</td>
<td>1.57±0.17</td>
<td>0.29 (0.13–0.46)</td>
<td>1.21</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Imidacloprid+TPP</td>
<td>1.68±0.20</td>
<td>0.29 (0.08–0.49)</td>
<td>1.21</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Imidacloprid+DEM</td>
<td>1.52±0.21</td>
<td>0.33 (0.11–0.58)</td>
<td>1.06</td>
<td></td>
</tr>
<tr>
<td>RF75</td>
<td>Imidacloprid</td>
<td>2.10±0.13</td>
<td>25.41 (22.80–28.80)</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Imidacloprid+PBO</td>
<td>1.95±0.25</td>
<td>7.28 (5.44–8.91)</td>
<td>3.49</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Imidacloprid+TPP</td>
<td>1.86±0.24</td>
<td>13.70 (9.59–17.30)</td>
<td>1.85</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Imidacloprid+DEM</td>
<td>2.04±0.28</td>
<td>23.90 (16.40–30.50)</td>
<td>1.06</td>
<td></td>
</tr>
</tbody>
</table>

a) Fiducial limits (probit analysis using SPSS data processing system). b) SR (synergism ratio) = LC50 of insecticide/LC50 of insecticide+synergist.
Table 3. PNOD, CarE and GST activities in the SS and RF75 strains of A. gossypii \(^a\)

<table>
<thead>
<tr>
<th>Strain</th>
<th>PNOD (±SE) (nmol/min/mgpr)</th>
<th>Ratio(^b)</th>
<th>CarE (±SE) (nmol/min/mgpr)</th>
<th>Ratio(^b)</th>
<th>GST (±SE) (µmol/min/mgpr)</th>
<th>Ratio(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS</td>
<td>4.27 (±0.32) a</td>
<td>4.59</td>
<td>0.90 (±0.06) a</td>
<td>2.33</td>
<td>16.50 (±0.09) a</td>
<td>1.13</td>
</tr>
<tr>
<td>RF75</td>
<td>19.60 (±1.81) b</td>
<td></td>
<td>2.10 (±0.05) b</td>
<td></td>
<td>18.60 (±0.22) a</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)Mean activity values in the same column followed by different letters are significantly different (Statistical analysis was performed by one-way ANOVA, and comparisons of the means were made by Fisher’s LSD test and t-test (p<0.05). \(^b\)Ratio= value of RF75 activity/ SS activity.

Discussion

The present results clearly indicate that the cotton aphids with resistance to imidacloprid showed obvious cross-resistance to the other neonicotinoid insecticides. Neonicotinoid insecticides are classifiable into three generations according to structural analysis, imidacloprid, acetamiprid and nitenpyram belong to the first generation of neonicotinoids with a heterocycle of chloropyridine; thiamethoxam and clothianidin belong to the second generation with a heterocycle of chlorinated thiazole; dinotefuran is a third generation with a heterocycle of tetrahydrofuran. The RF75 strain showed the highest level of resistance to all three generations of neonicotinoid compounds (>10-fold). The low levels of cross-resistance to the first-generation neonicotinoids (acetamiprid and nitenpyram) (<6-fold) also have been described at the 27th (24-fold) and 45th (41.7-fold) generations for the imidacloprid-resistant strain of A. gossypii in our laboratory in previous studies. However, the imidacloprid-resistant strain of A. gossypii at the 45th generation did not develop cross-resistance to the second (thiamethoxam, clothianidin) or third (dinotefuran) generation of neonicotinoid compounds.

Yang et al. have reported that the imidacloprid-resistant strain of A. gossypii at the 27th generation exhibited obviously negative cross-resistance to pymetrozine (0.28-fold). However, the RF75 strain showed 5.28-fold resistance to pymetrozine in this study. The mechanisms of cross-resistance to pymetrozine by the more imidacloprid-resistant strain of A. gossypii need to be further investigated. Additionally, the present results indicate that the RF75 strain showed different levels of cross-resistance to insecticides (5.90–9.18-fold) having target sites other than nAChR. These results suggest that A. gossypii has a potency to develop a high level of resistance to imidacloprid and exhibit cross-resistance not only to neonicotinoid insecticides targeting nAChR, but also to other insecticides with different modes of action.

In general, imidacloprid resistance and cross-resistance mainly result from metabolic detoxification of esterases and cytochrome P450, mutation at an insecticidal target site and other factors such as reduced penetration of the insecticide through the cuticle. The increase in activities of esterases and cytochrome P450 had been reported to be one of the most important mechanisms for imidacloprid resistance in many insects. However, reduced penetration of an insecticide through the insect cuticle is not considered an important resistance mechanism. In this study, PBO and TPP showed an effect on imidacloprid toxicity in the RF75 strain, with a synergism ratio of 3.49 and 1.85, respectively. Furthermore, the activities of PNOD and CarE were significantly higher in the RF75 strain than in the SS strain. These results suggest that cytochrome P450 and CarE probably are important factors related to imidacloprid resistance in the RF75 strain.

Gorman et al. have found that cytochrome P450 may be the major cause of the cross-resistance between neonicotinoids and pymetrozine in Bemisia tabaci. The important metabolic detoxification of CarE for other insecticides such as pyrethroids, organophosphates, and carbamates, has also been proven in the cotton aphid. Therefore, a possible explanation for the multi-resistant background of the RF75 strain might be the increased activities of CarE and cytochrome P450. Nevertheless, the present results demonstrate that A. gossypii can develop a high level of resistance to imidacloprid, and this may also result in more serious cross-resistance problems.

Acknowledgements

This research was supported by the National Natural Science Foundation of China (No. 31301695), the Scientific Research Award for Outstanding Young and Middle-Aged Scientists in Shandong Province (No. BS2012SW015), the Project of the Shandong Province Higher Educational Science and Technology Program (No. J13LE18), and the earmarked Fund for Modern Agro-Industry Technology Research System in Shandong Province.

References

10) K. Gorman, G. Devine, J. Bennison, P. Coussons, N. Punchard and I.